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PROGRESS REPORT

INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

1 January through 31 March 1966

R. G. Lindberg, Ph.D.
Principal Investigator

PREPARED UNDER CONTRACT NASw-812

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OFFICE OF SPACE SCIENCES
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
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HIBERNATION CHARACTERISTICS IN PEROGNATHUS LONGIMEMBRIS

Kyllikki Grubel

I. INTRODUCTION

It has been established that Perognathus longimembris has a circadian rhythm of torpidity throughout the year (Bartholomew et al., 1957, Chew et al., 1965). The pocket mice are able to go into torpidity and arouse spontaneously every 24 hours. They also may stay in torpidity for a few days without arousing when kept in a cold environment (+10°C).

The present work was designed to determine whether there is a seasonal factor influencing the torpidity pattern of the pocket mice and whether their torpidity is comparable to the hibernation phenomenon of the "classical" hibernators. During the first part of arousal, the true hibernators maintain a differential vasoconstriction in order to enhance the rapid rewarming of the brain and the vital organs in the thorax. Their brown fat performs a thermogenic role during arousal. And in most hibernators, the thyroid glands are involuted during hibernation season. This report summarizes the results of experiments in progress designed to establish whether these systems are present in P. longimembris and whether there are seasonal variations in these parameters.

II. MATERIALS AND METHODS

The animals used for the present work were randomly chosen individuals of the species P. longimembris. The age of the animals varied from 1½ to 3½ years, and both males and females were used. During their captivity the mice had been kept in individual one-gallon jars, which contained about one inch of sand. Ambient temperature was held constant at ca. +22°C, and 12 hours light-dark photoperiod was provided. The mice were fed a mixture of rolled oats, bird seed and sunflower seed ad libitum and an occasional piece of lettuce or apple, but no water. All the mice had been captured near Whitewater, California.

A. Blood flow rate and colonic temperature during arousal

1) Apparatus. A scintillation probe (Nuclear-Chicago DS 5), which was connected to a rate-meter (Nuclear-Chicago 1620 B) and further to a recorder (Nuclear-Chicago R 1000), was placed in a vertical position underneath a working table. On the table above the scintillation probe was a standard size (5 x 10 x 20 cm) lead brick, with a 5 mm diameter hole drilled through its center. The lead brick was topped by a fitted pine-wood board.

2) Method. When an experiment was started, a mouse was removed from its jar, and tied onto the board ventrally in such a position that the left thigh was directly above the 5 mm hole in the lead brick. A thermistor probe was placed in the colon (ca. 2 cm deep) to obtain a continuous record of the body temperature. 2 μc of I^{131} in 0.2 - 0.5 ml of 0.9% NaCl solution was then injected into the musculature of the left thigh above the scintillation probe. The recorder thus provided a continuous record of I^{131} clearance from these peripheral muscles, thereby giving an indication of the blood flow rate at site.

In handling the data obtained from these experiments, time zero was chosen to be the time of iodine injection. To achieve the temperature records (Fig. 1) a reading was taken from the original records at 5 min. intervals. The iodine clearance record in the arousing mice formed a two-part curve. The first part indicated negligible blood flow. The second part showed abrupt increase in blood flow rate. The point of change was taken to indicate the length of time that peripheral vasoconstriction was maintained.

3) Animals. One group of nine mice was kept in normal room temperature and used for controls. Two groups of mice were placed in a constant temperature room with a temperature of +10°C and a 12-hour light-dark cycle. The fall group (October, 6 mice) had food at will. The winter group (February, 9 mice) was first given food, but since after four days of cold exposure only one animal had become torpid, the food and sand were removed from the jars. The animals then became torpid, with the exception of one mouse that died.

B. Amount of brown fat

For this study, each mouse was first weighed and then anesthetized with ether. All brown fat was removed from the mouse and weighed immediately. From these figures, the brown fat percentage of total body weight was calculated.

Two groups of mice were studied: 1) a group of ten pocket mice kept in normal room temperature, with food ad libitum; 2) a group of ten mice kept at +10°C, with food ad libitum for three to six days before sacrifice.

C. Histological appearance of the thyroid glands

For these observations, the same individual mice were used as for Part B, brown fat measurements. Due to the small size of P. longimembris, the removal of the thyroid glands was performed under the microscope. Also, in the subsequent handling of these tissues the small size called for special methods. When the tissues were embedded in paraffin, the following steps were taken: the thyroid glands were embedded in the ordinary manner into colorless paraffin in a mold 3 x 3 x 2 mm. The aluminum foil was then peeled off the paraffin, and the small paraffin cube with the thyroid glands in it was subsequently embedded into colored paraffin in a regular size mold.

The tissues were sectioned 6μ thick and stained with hematoxylin and eosin. The diameter of the follicles and the thickness of the epithelium were measured.

III. RESULTS

A. Colonic temperature and peripheral vasoconstriction during arousal

The results of these experiments are illustrated in Figures 1 and 2. The colonic temperature of the control mice stayed between +32°C and +36°C. No peripheral vasoconstriction was observed in these mice.

The arousal of the torpid mice in October forms a colonic temperature curve characteristic of hibernating animals. There is definite vasoconstriction in the peripheries, the average time being 38.3 min. The fastest arousing mouse released the peripheral blood flow 15 min. after beginning of the experiment. The mouse with the longest arousing time maintained this vasoconstriction for 65 min.

However, in February when the mice had to be starved before they would become torpid, only one out of eight mice aroused. The average time

of vasoconstriction was only 11.2 min. The only mouse that did arouse, rewarmed at about the same rate as the slowest arousing mouse in October. This mouse maintained the peripheral vasoconstriction for 10 min.

B. The amount of brown fat

The body weights, brown fat weights and the percent of brown fats relative to body weights are shown in Figure 3. As is seen in this figure, the differences in the body weights and the absolute brown fat weights are not significant. But the relative amount of brown fat is significantly larger in the cold exposed mice. The amount of brown fat in the control mice was 2.0 $\pm$ 0.9% and in the group of cold exposed mice 3.3 $\pm$ 0.4% of body weight.

C. The thyroid glands

The histological appearance of the thyroid glands is different in the two groups of mice. In the control group, the follicles are large (diameter 59.5 $\pm$ 11.7μ) and filled with colloid. The epithelial cells are squamous, the average thickness of the epithelium being 4.5 $\pm$ 0.8μ. In general, the thyroids of the control group in the latter part of January may be described as involuted.

On the other hand, the thyroid glands of the cold exposed mice at the same time of the year appeared very active. The center parts of the glands were not organized into follicles. The cells were crowded, and little or no colloid was present. The remainder of the gland consisted of small follicles with the average diameter of 38.9 $\pm$ 6.1 μ. The epithelial cells were cubical or cylindrical in shape, and the thickness of epithelium was greater than in the control group, 9.9 $\pm$ 1.7 μ. The data are illustrated in Figure 4. It shows that the two groups obviously have differences in their thyroid gland histology.

IV. DISCUSSION

The purpose of this study was to determine whether the physiology of torpor in P. longimembris is similar to the physiology of "classical" hibernators. Obviously, the data available at the present stage do not permit any conclusive deductions. But they do suggest (1) that P. longimembris does behave as a "classical" hibernator, and (2) that there may be a seasonal rhythm in the torpidity pattern of P. longimembris. The arousal pattern and simultaneous peripheral vasoconstriction seen in

these mice in October confirm the hypothesis that the organism would have the same types of physiological mechanisms as has been observed by previous authors (Bullard et al., 1962, Lyman et al., 1963) in the true hibernators. The fact that they seem to be less apt to go into torpidity, are unable or very slow to arouse and maintain their vasoconstriction for a relatively short time when trying to arouse in early February is possibly due to the late season. Bartholomew et al. (1957) state that the potentiality for hibernation (or aestivation) in P. longimembris presumably exists throughout the year, and that there is no difference in arousals during the year. But he did his experiments between June and September and aroused the animals in room temperature, whereas our arousal experiments were conducted in an ambient temperature of +10°C. These differences in the experiments would account for the different results. The possibility of seasonal effects on the torpidity pattern of P. longimembris has been previously suggested by Chew et al. (1963). They found that P. longimembris kept at +10°C for nine months on a constant 12-hr photoperiod showed the greatest incidence of torpidity in the winter. The works of other authors have pointed towards the possibility of peripheral vasoconstriction during arousal in pocket mice. Eisenberg (1963) says that during arousal, coordination is attained first in the forelimbs while control over the hindquarters lags some minutes behind. Bartholomew et al. (1957) found that rectal temperature lagged slightly behind the oral temperature during arousal. Our results seem to confirm these statements.

Several authors have stated that the thyroid glands are involuted during hibernation (e.g., Kayser, 1961; Hoffman, 1964). The appearance of the thyroid glands of the control group in late January seem to agree with this. The thyroid glands of the cold exposed mice appeared more active. This might be explained by the late season when the mice would tend to maintain their normal body temperature rather than go into hibernation under exposure to the stress of a cold environment. But if this would be the case, it would mean that there is some difference in the system of hibernation and aestivation. This would be the opposite of what Bartholomew et al. (1957) states. There is disagreement among various authors as to the state of the thyroid glands during hibernation, and Kayser (1961) concludes: "Hibernation is usually accompanied by an

underfunctioning of the thyroid, but small sized hibernators may hibernate even though their thyroids show the morphological signs of active glands and though their blood contains active forms of iodine elaborated by the thyroid."

The importance of brown fat to hibernators during their arousal has been shown by Smith and Hock (1963) and Kauppinen et al. (1964, abstr.). Our data on the amount of brown fat in P. longimembris show that these mice have a relatively large amount of brown fat and that their brown fat readily responds to cold stimulus.

V. SUMMARY

1) The arousal from torpidity was studied in P. longimembris in October and early February. During arousal, the colonic temperature and the blood flow rate in a hind limb were observed. In October, the temperature increment formed a curve, which is typical of "classical" hibernating mammals. The blood flow rate in the hind limb indicated peripheral vasoconstriction. In February, seven out of eight mice failed to arouse. Their peripheral vasoconstriction was maintained for a relatively short time, if at all.

2) The amount of brown fat and the histological appearance of the thyroid glands were studied in late January. The amount of brown fat in control animals was 2.0% of body weight. In cold exposed mice, it was as much as 3.3%. The thyroid glands in control mice appeared passive and in cold exposed mice, active.

3) The sparse data do not allow definite conclusions, but the results of these experiments suggest that the physiology of torpor in P. longimembris is similar to that of "classical" hibernators, and that there is a possibility of a seasonal factor affecting their torpidity pattern.

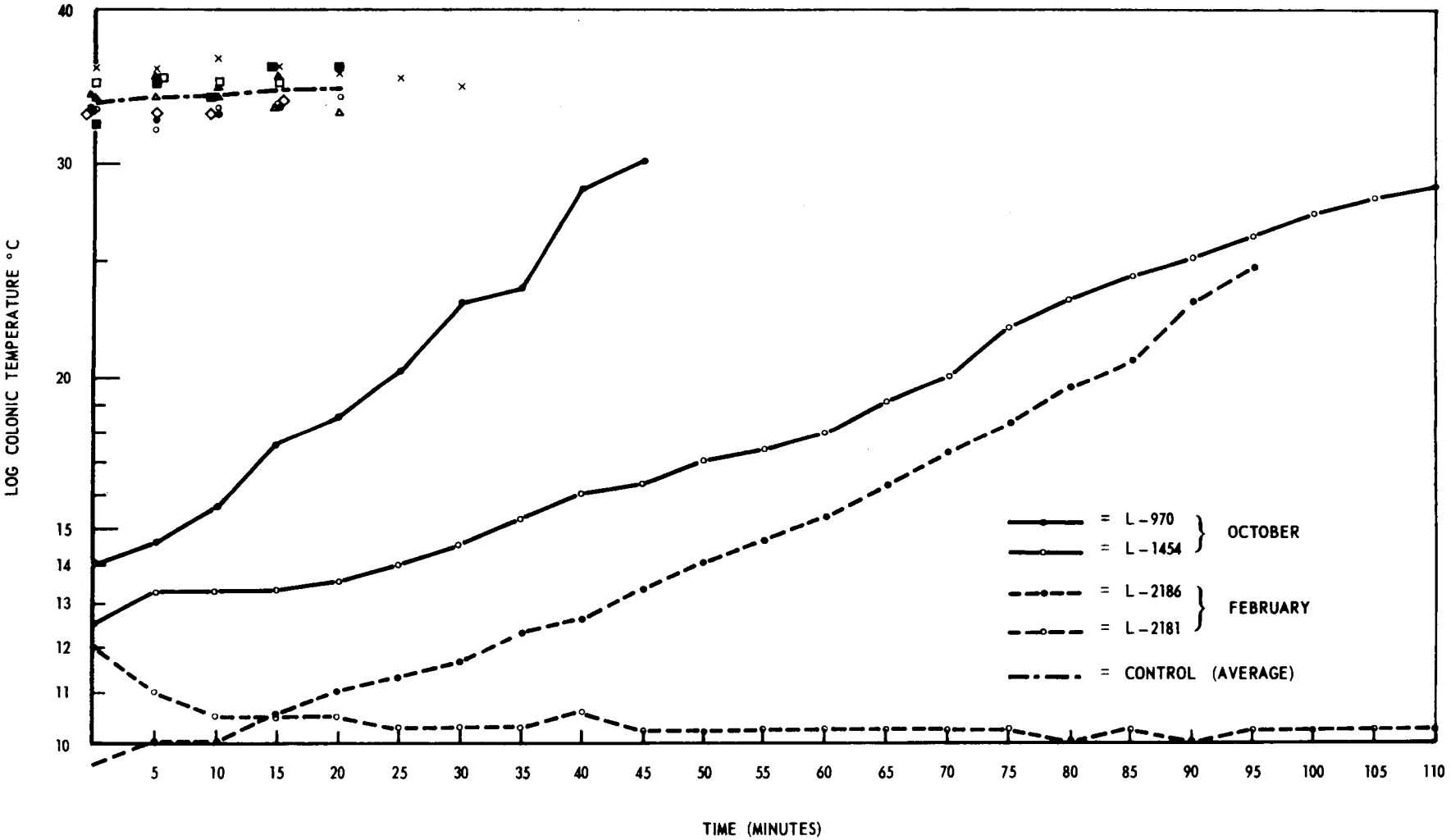

Figure 1. Rate of body warming during arousal in Perognathus longimembris.

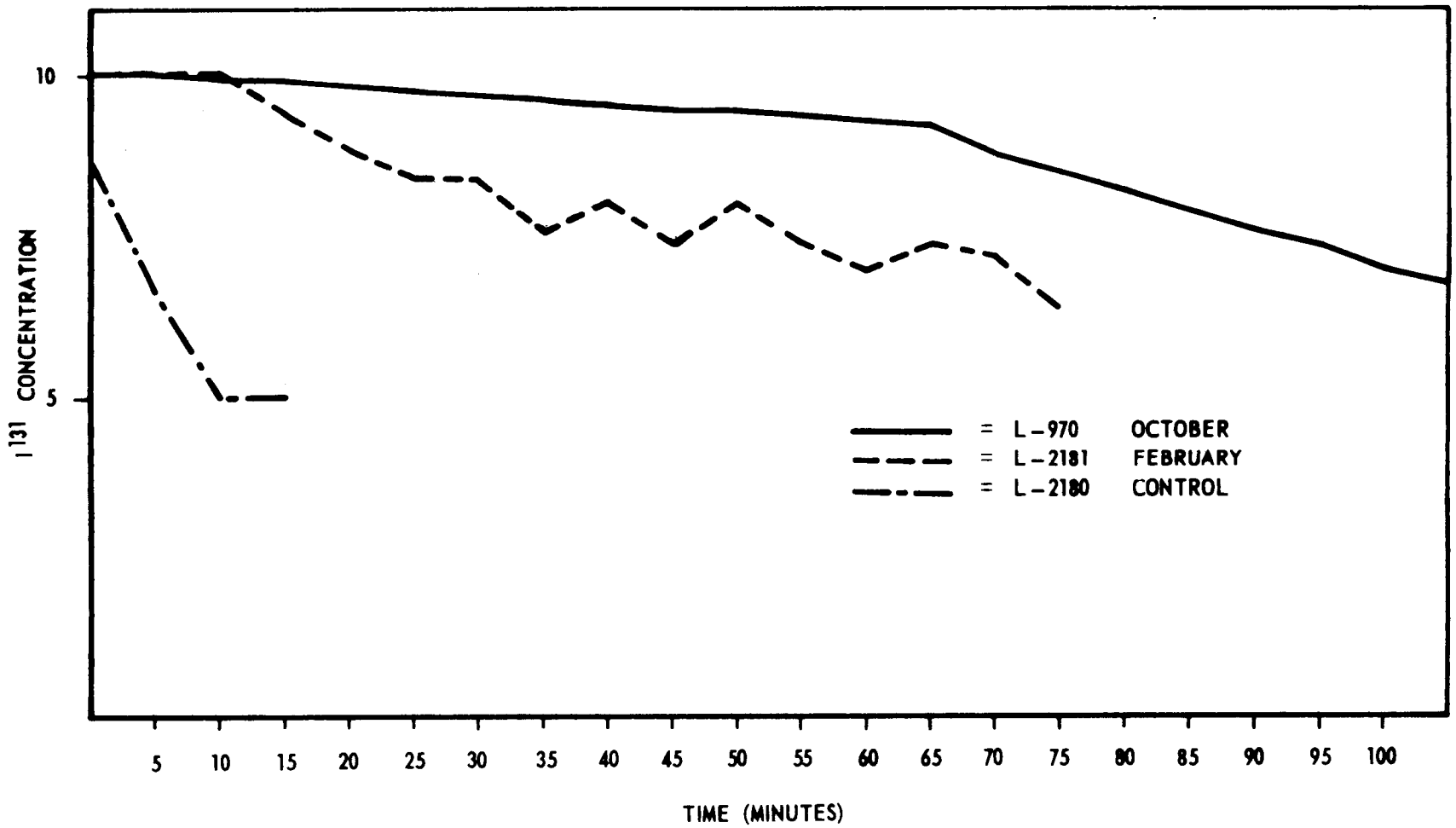

Figure 2. Vasoconstriction in Perognathus longimembris as demonstrated by clearance rate of I^{131} from hind limb muscles.

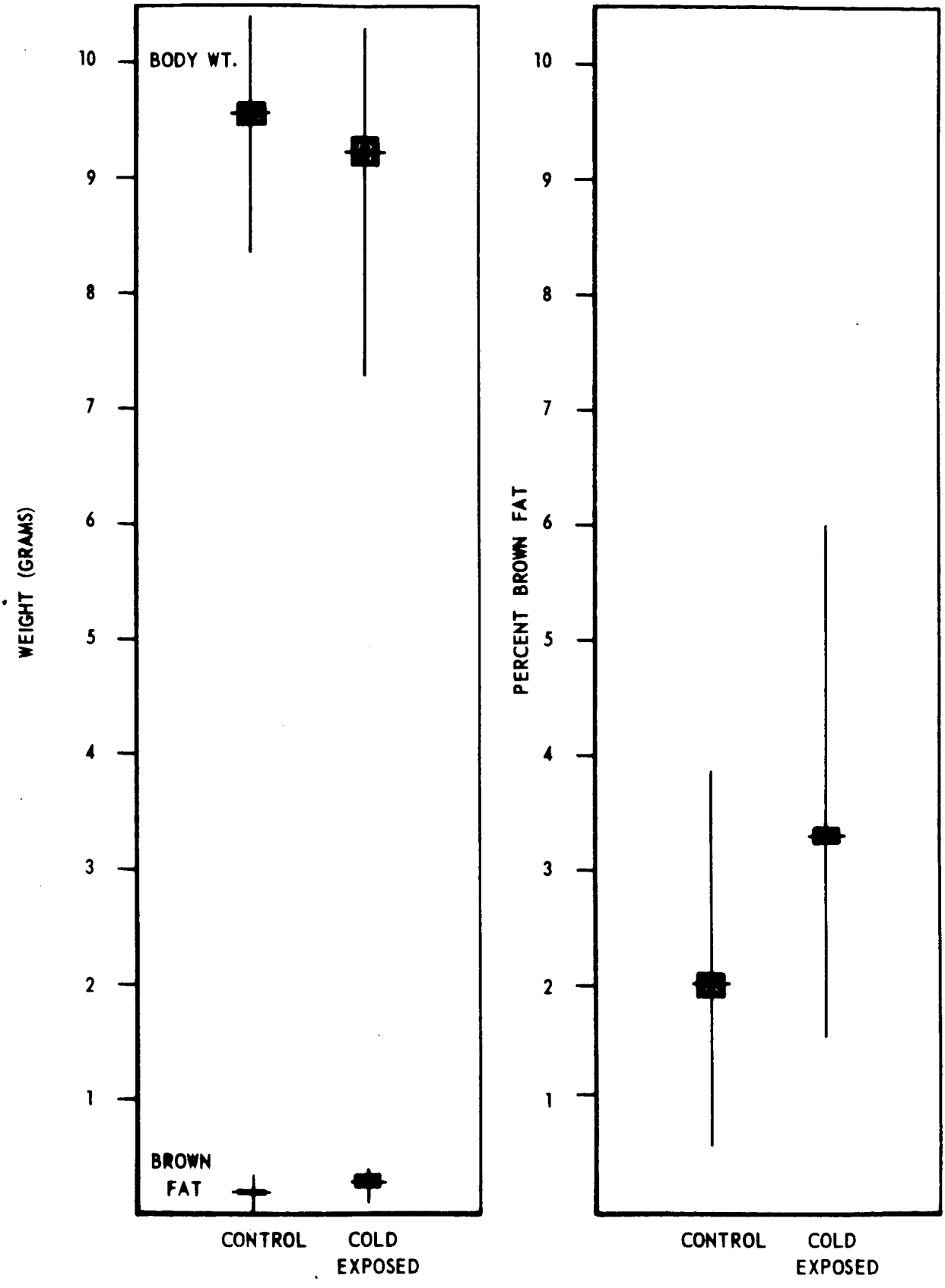

Figure 3. Proportion of brown fat to body weight in Perognathus longimembris (10 mice/group).

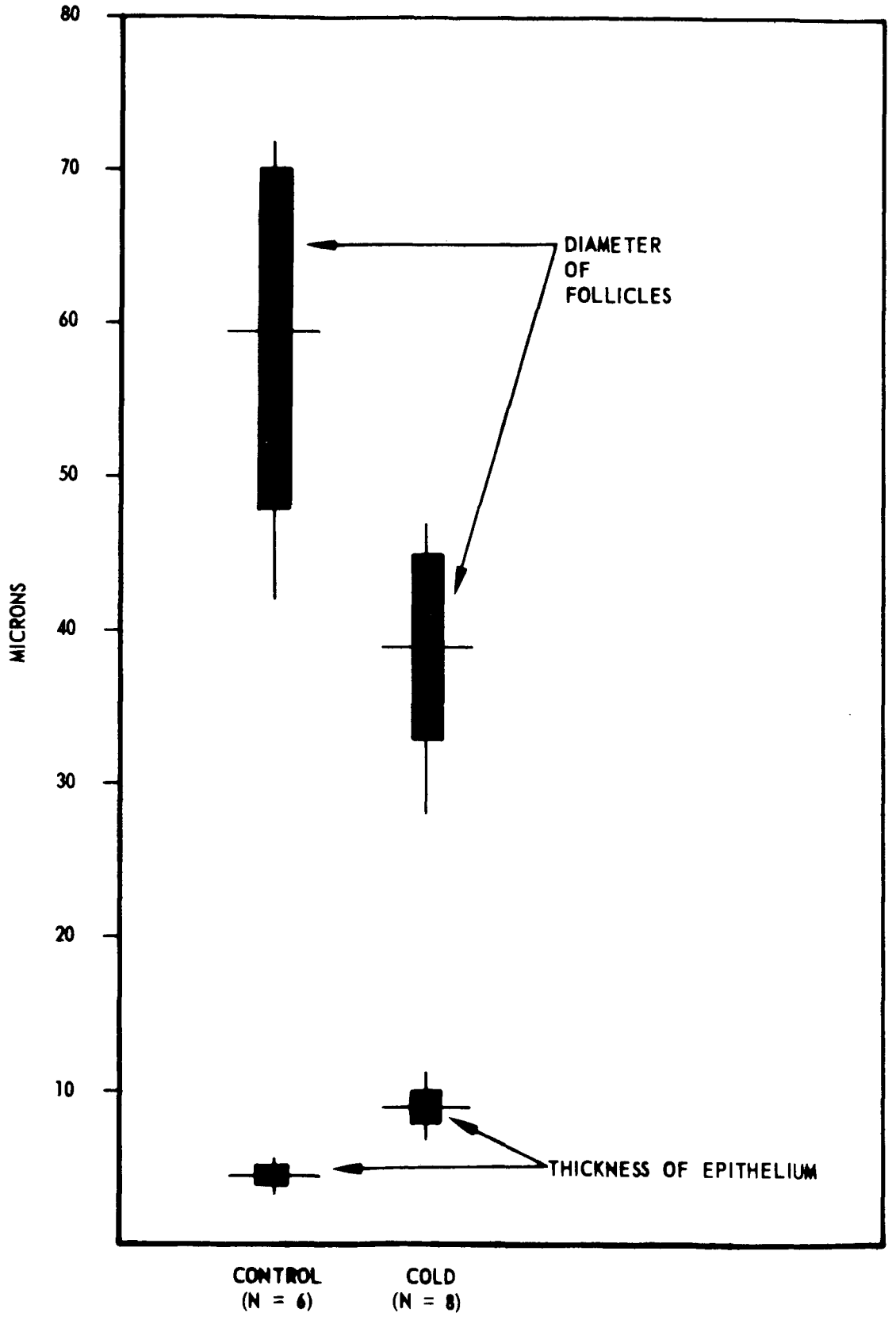

Figure 4. Thyroid gland structure in cold exposed and control groups of Perognathus longimembris.

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PRELIMINARY REPORT OF FREE RUNNING RHYTHMS OF BODY TEMPERATURE IN PEROGNATHUS LONGIMEMBRIS AT 22°C AND 10°C

Page Hayden

I. INTRODUCTION

The study of hibernation has both a physiological and a temporal facet. The yearly life cycle of a "typical" hibernator involves three major segments: 1) preparation for hibernation, 2) hibernation, and 3) arousal and reproduction. During the hibernation period, the typical hypothermic state is broken periodically, and the body temperature is raised to normal. These arousals occur in a rhythmic manner every few days or weeks, depending on the species and environmental conditions. These rhythmic arousals with immediate re-entry into the hibernating state are different phenomena from the overall yearly cycle of hibernation vs. normal activity.

The Little Pocket Mouse (Perognathus longimembris) undergoes cyclic periods of depressed metabolism of a circadian nature (Chew, Lindberg and Hayden, 1965) and also an annual cycle of increased expression of this rhythm in any given number of animals (unpublished observation in the laboratory) which corresponds with field observations. In nature, these animals disappear from above ground activities from October until late January and have been thought to hibernate. It was the purpose of this phase of the study to document the metabolic rhythm of torpor and activity under conditions more closely resembling those found in nature than previously used. The most pertinent factors are: time of year, surplus of food, constant dark, isolation from noise and low temperature.

II. METHODS AND MATERIALS

The experimental plan was to constantly monitor the body temperature of a group of pocket mice that were to be maintained in dark at three temperature levels (22°C, 10°C and 5°C) for three weeks at each level. Temperature monitoring telemeters were surgically implanted within the peritoneal cavity and were not tied to any organ. The animals were allowed to recuperate from the implantation for 8 days, by which time the incision had healed, and the animal appeared to be normal. They were then held for 7 days at 22°C with a 12 hour light (0600-1800) and 12 hour dark (1800-0600) photoperiod. Animals used in this experiment were selected because frequent observations indicated a tendency to periodic torpor.

All experimental chambers were provided with a half-inch substrate of desert sand, 35 gm of sunflower seeds and a handful of dry grass for bedding. The individual animal chambers were placed in a light-proof constant temperature room and were semi-isolated from each other by open front boxes constructed of acoustical tile. After 29 days of continuous isolation, the food supply was replenished and a small amount of dry grass added. Entry into the constant temperature room was made with the aid of a ruby red light (photographic safe light) and care was taken to keep direct illumination of animals at a minimum. Previous experience has indicated that Perognathus cannot entrain to this portion of the spectrum or intensity of illumination. Total entry time was about 7 minutes.

The animals were kept at 5°C longer than the allotted time period. However, because of multiple failures of a portion of the data recording system, unreliable data were obtained during the 5°C portion of the experiment. It is significant, however, that the animals at 5°C did undergo periodic torpor and survived the 36-day exposure.

III. RESULTS

Two animals were found dead when the chamber was entered on the 29th day. One animal had escaped from its monitoring chamber and presumably had starved. The other animal was dead in its monitoring chamber, with no obvious cause of death.

Food consumption: At the termination of the experiment, the remaining five mice appeared to be in excellent health, even though a general weight loss was noted. All animals lost weight, with an average of 1.3 gm (range 0.8 - 1.8 gm). Food consumption averaged 21.1 gm (range 14.8 - 23.8 gm) with three of the five consuming approximately 23.7 gm of sunflower seeds during the 86 days of the total experiment. These range values of 0.17 - 0.27 gm food/day are 1/3 to 1/5 of the amount required by the animals to maintain normal body temperature.

Sequence and duration of torpor: For the purpose of this paper, torpor is defined as the decline in body temperature to within one or two degrees above the ambient temperature, 22°C and 10°C respectively. The onset and arousal from torpor during a 24-hr period are plotted in Figure 1. Periods of torpor were observed initially during the light portion of the regimen and were generally evident by the fourth or fifth day. After the constant 24D was initiated on the 7th day, all animals exhibited daily periods of

torpor. These torpor periods lasted from four hours to the entire 24-hr period. In some cases, multiple periods of torpor were observed (#5 on day 30, Fig. 1).

The duration of sequential periods of torpor is plotted in Figure 2. It appears that the periods of torpor progressively lengthen until about the 8th or 9th day; however, one animal reached a plateau after four periods of torpor (#3, Fig. 2). The maximum time spent in continuous torpor was 4200 minutes (72 hours) at 10°C. One animal exhibited five sequential periods of torpor of over 3700 minutes (61 hours) each. At 22°C, 69% of the torpor periods were from 200 to 800 minutes (3-13 hours), with 30% being from 400 to 600 minutes (6.6-10 hours), and 9% were from 1800 to 2200 minutes (30-36 hours). At 10°C, 24% of torpor periods were from 200 to 800 minutes, 32% were greater than 2200 minutes (= max time in torpor at 22°C) and 11% were from 3600-4200 minutes (60-70 hours).

A decrease in ambient temperature and concomittant decrease in the ultimate body temperature during torpor does not initially increase the length of torpor exhibited by the animal. Four of the five animals reacted to the decrease in ambient temperature (from 22°C to 10°C) with a <u>decrease</u> in duration of torpor (compared to the duration of torpor at 22°C). This decrease in duration of torpor is evident for 3-5 torpor periods after the temperature change. Two animals (Fig. 2, #3 and #7) were unusual in that they underwent two lengths of torpor periods, approximately 600 minutes and 1600-2000 minutes, during the 22°C ambient temperature. The long periods were generally separated by one or two short periods. One of these animals (#3) was unique in the relatively long torpor period that was maintained during both the 22°C and 10°C temperature regimens. This long period, however, was evident less frequently at 22°C than at 10°C. At the latter temperature, it was the daily mode. Another animal (#6, Fig. 2) maintained a relatively constant short duration of torpor in both 22°C and 10°C. In general, as was expected, the duration of torpor was prolonged in 10°C as compared to 22°C.

The times of entry and arousal from torpor are distinctive points in the life processes of this animal and can be used as phase markers of the metabolic circadian rhythm. In the 22°C temperature regimen, entry and arousal from torpor had a strong, well-defined rhythm (e.g., Fig. 3).

Several days after the termination of the 12L-12D light regimen and commencement of constant dark, all entered into free-running metabolic rhythms. These free-running periods varied from 22 hrs to 23 hrs and 41 minutes. The arousal from torpor seemed to be a more predictable and consistent marker than entry at ambient temperature of 22°C. The temperature drop to 10°C on day 29 apparently caused a severe disturbance in the pattern of a stable periodicity. One animal (Fig. 4) regained a well-defined stable pattern after showing a disturbance for about seven to eight days. Several animals changed from a less than 24-hr rhythm to a greater than 24-hr rhythm. With one animal, it was not manifested until five days after the temperature change and was a transient phenomenon lasting about seven days.

The arousal of an animal at a time in phase with the established circadian rhythm after one or two days in a continuously hypothermic state was a rather common occurrence, and in some animals had an amazing accuracy (Fig. 1, #4, 10°C, body temperature = $\backsim$11°C).

Table I is a summary of free-running periods calculated from both entry and arousal from torpor and subjective estimates of the accuracy of the rhythm by the two phase markers. Transients of rhythms induced by the temperature decrease are evidenced by polyphasic nature of free-running period and by gross changes in length of period. In all but one individual there was a degradation of accuracy of the rhythm.

IV. DISCUSSION

This study again emphasizes the metabolic lability of P. longimembris (Chew, Lindberg and Hayden, 1965). Prolonged periods of natural torpor were evidenced when the animal was not stressed, i.e., food was provided in excess at all times, natural substrate and bedding material available, a reasonable degree of isolation, temperature relatively high and normal gaseous atmosphere. The metabolic lability probably reflects the seasonal cycle of hibernative behavior of this species, although it appears in some animals throughout the year. The experiment was carried out during that portion of the year when mice in the field are absent from activity above ground (i.e., cannot be trapped) and presumably are undergoing periods of reduced metabolism (Chew and Butterworth, 1964).

	22°C		10°C	
	Entry	Arousal	Entry	Arousal
#3	-good- τ = 22 hr 27 min	-excellent- τ = 22 hr 27 min	-poor- very erratic	-fair- (2 phases) τ = 22 hr 27 min τ = 24 hr 8 min
#4	-good- 1st 12 days τ = 24 hr after 12 days τ = 22 hr 52 min	-excellent- 1st 9 days τ = 24 hr after 9 days τ = 23 hr 30 min	-excellent- τ = 22 hr 34 min	-excellent- τ = 22 hr 10 min
#5	-good- τ = 22 hr 40 min	-good- τ = 23 hr	-fair- τ = 24 hr 19 min	-fair- τ = 24 hr 19 min
#6	-fair- τ = 23 hr 16 min	-good- τ = 23 hr 41 min	-good- (2 phases) τ = 22 hr 41 min τ = 23 hr 24 min	-poor- 1st half -good- 2nd half τ = 23 hr 35 min
#7	-good- 1st 9 days τ = 24 hr after 9 days erratic τ = ~ 22 hr	-good- 1st 9 days τ = 24 hr after 9 days erratic τ = ~ 22 hr	-good- (3 phases) τ = 23 hr 30 min τ = 26 hr 24 min τ = 23 hr 42 min	-good- more erratic but same form

Table 1. Summary of P. longimembris free-running period at two different ambient temperatures. Period length and subjective estimate of accuracy based on entry and arousal from torpor.

The duration of individual torpor periods (hibernation) was longer in this experiment than has been observed in previous experiments. The experimental conditions of isolation from periodic noise, constant dark, surplus food, sufficient time to acclimate and time of year probably contributed to the maximum time spent in torpor. The observed maximum of 70 hrs may represent the limits of natural continuous hypothermia that this small mammal can undergo at an ambient temperature of 10°C. It was unfortunate that very little data were derived from the 5°C portion of the experiment, but there was a strong indication that duration of torpor was increased, and the circadian component of arousal was still in operation at this temperature. The weight loss of the animals was greater than expected but did not appear to affect the general well being of the animals. At the termination of the experiment, all had sleek coats, bright eyes, showed normal activity and are still living. It is possible that there was preferential use of body fat as an energy source, even though food was available at all times. It is impossible to tell if the weight loss was gradual or if it was lost incrementally within the three temperature regimens.

When a typical hibernator (ground squirrel) enters hibernation, it undergoes periods of body temperature depression known as "test-drops". These drops occur over a period of a few days to weeks and are characterized by each drop being slightly lower in temperature than the previous one. It is thought that these drops are a kind of acclimation of metabolic processes associated with hibernation and arousal (Strumwasser, 1960).

The pocket mice used in this experiment probably had undergone test drops necessary to go torpid in an ambient temperature of 22°C. It is interesting to note, however, that in most cases there was a sequential increase in duration of torpor at the beginning of the experimental period, and this may represent a kind of temporal "checkout" of prolonged hypothermic metabolism and functioning of arousal processes with time.

The change from 22°C to 10°C generally did not immediately increase the duration of torpor, but decreased the time in torpor for several days. The first torpid period of one animal (Fig. 2, #5) was characterized by a series of entries and arousals from torpor as if the animal's temperature dropping below a critical level immediately aroused the animal to normal

body temperature. It is possible that these were "test-drops" to acclimate the animal to the new low temperature.

It has been suggested in the literature (Twente and Twente, 1965) that duration of hibernation is a direct function of body temperature during hibernation. The arousal from hibernation might be initiated by a build up of specific metabolites (Pengelley and Fisher, 1961) as the rate of metabolism is governed by the temperature of the tissues. If this is true in pocket mice, it is difficult to explain how the duration of torpor could be increased from 4 to 6 times in some animals, when the temperature was decreased from 22°C to 10°C, and yet in other animals the duration increased only 2 times. One animal (Fig. 2, #3) had a duration of torpor in 22°C that was more typical of that shown in 10°C. This would indicate that the animal could either limit the production of the specific metabolites involved with arousal or could regulate the threshold of the metabolite sensor(s). The duration of hypometabolism does not seem to be a direct function of when the animal arouses, as evidenced, for example, by animal #4, Fig. 1, day 30-49. It apparently made little difference if the animal was torpid for one day or two days, for arousal occurred at the appropriate time with regard to the previous arousal. The presence of a circadian rhythm in this species has been documented (Chew, Lindberg and Hayden, 1965), and the present data indicate that the rhythm functions during extended periods of torpor with body temperatures of 11°C.

V. SUMMARY

1. Perognathus longimembris undergo daily periods of torpor even when not stressed, i.e., food plentiful and temperatures relatively high.

2. A continuous torpor of about 3 days was observed at 10°C and may represent a maximum for this species at this temperature.

3. A period of increasing duration of torpor was observed at the beginning of the experiment and may represent temporal "test-drops".

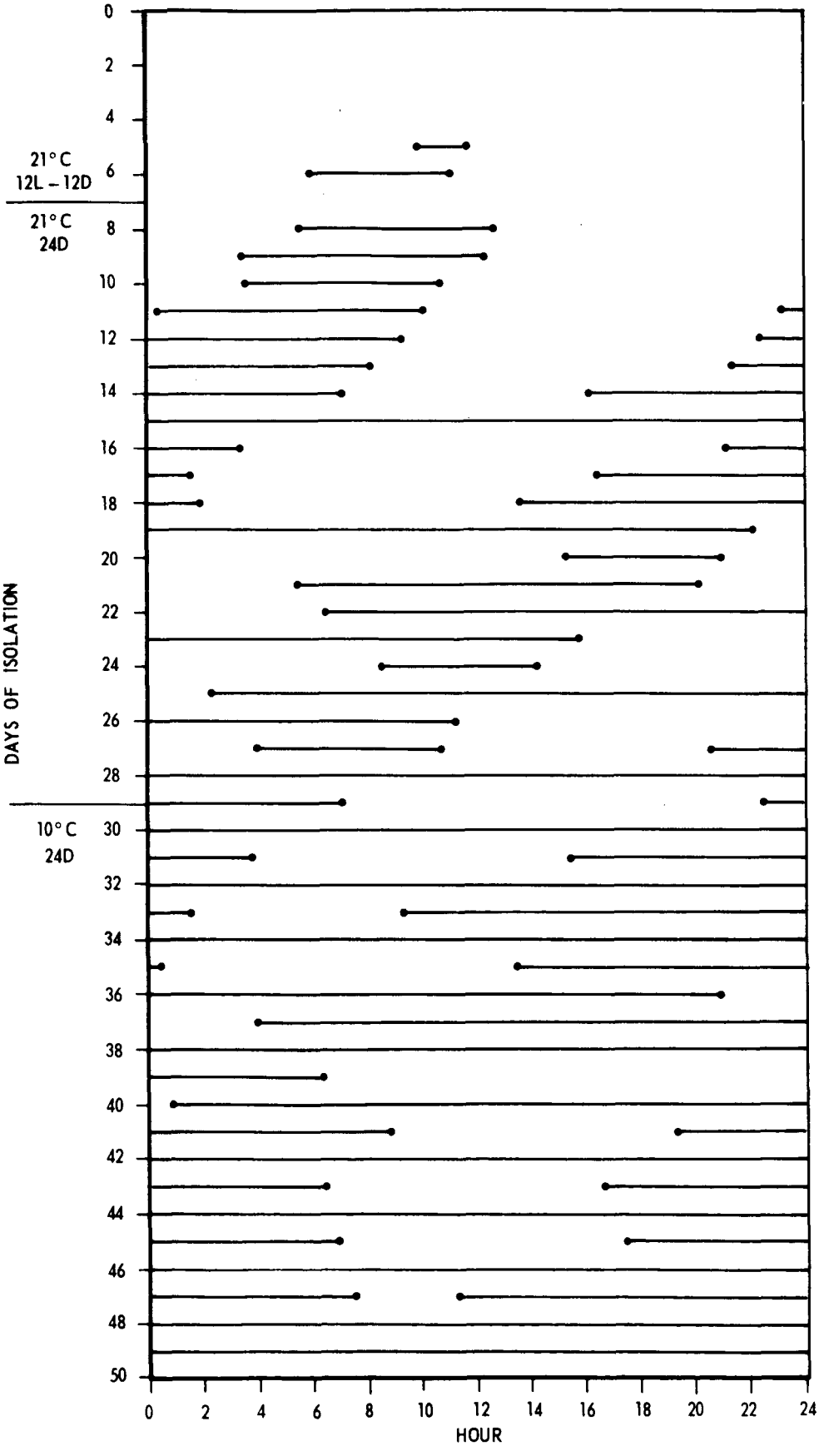
4. An ambient temperature decrease of 12°C initially decreased the duration of torpor.

5. At an ambient temperature of 10°C, the duration of torpor was from 2 to 6 times longer than at 22°C.

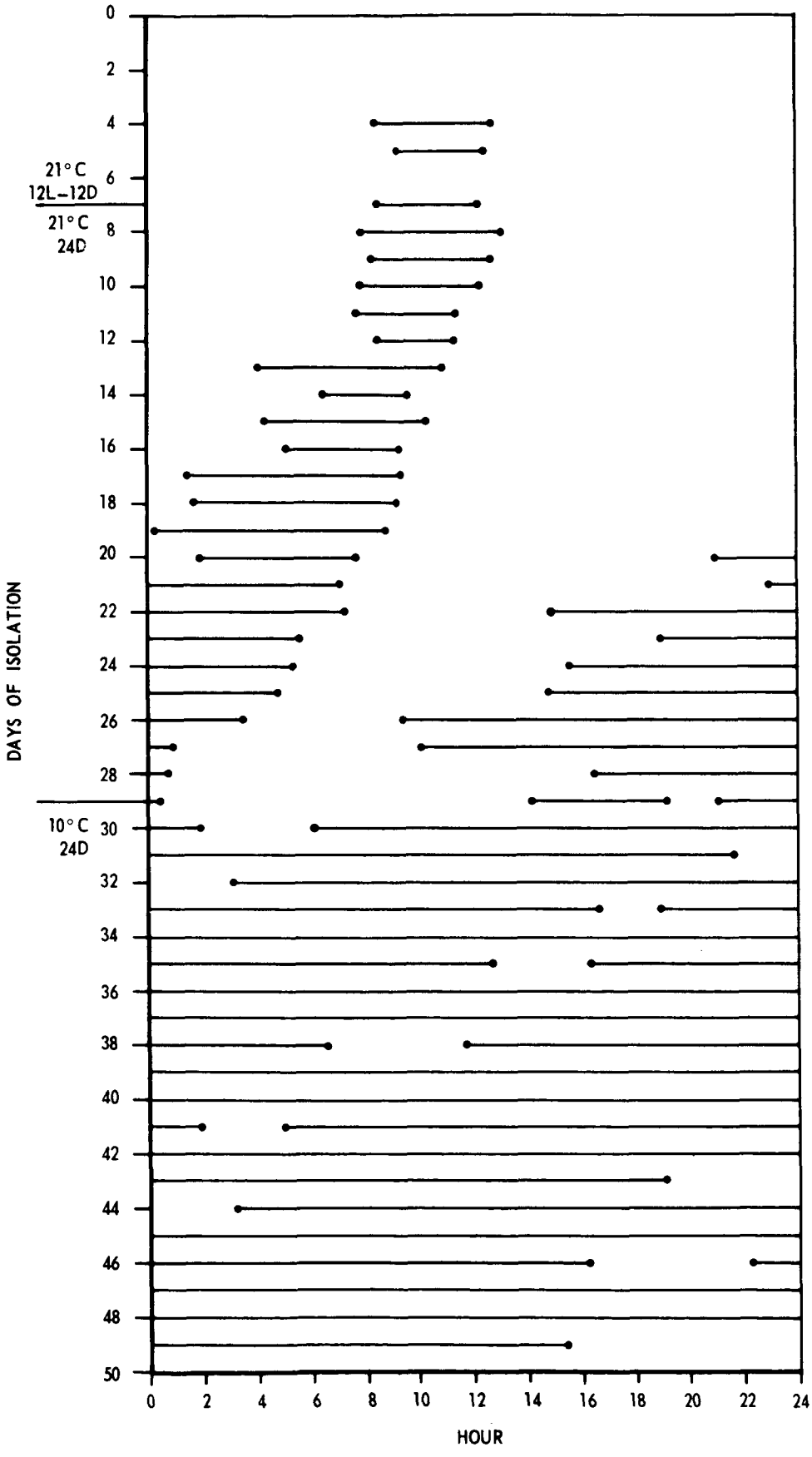
6. A strong circadian rhythm is involved in the time of arousal from a deep torpor (hibernation).

7. The temperature change resulted in a disturbance of the circadian rhythm and was, in general, less precise at the lower temperature.

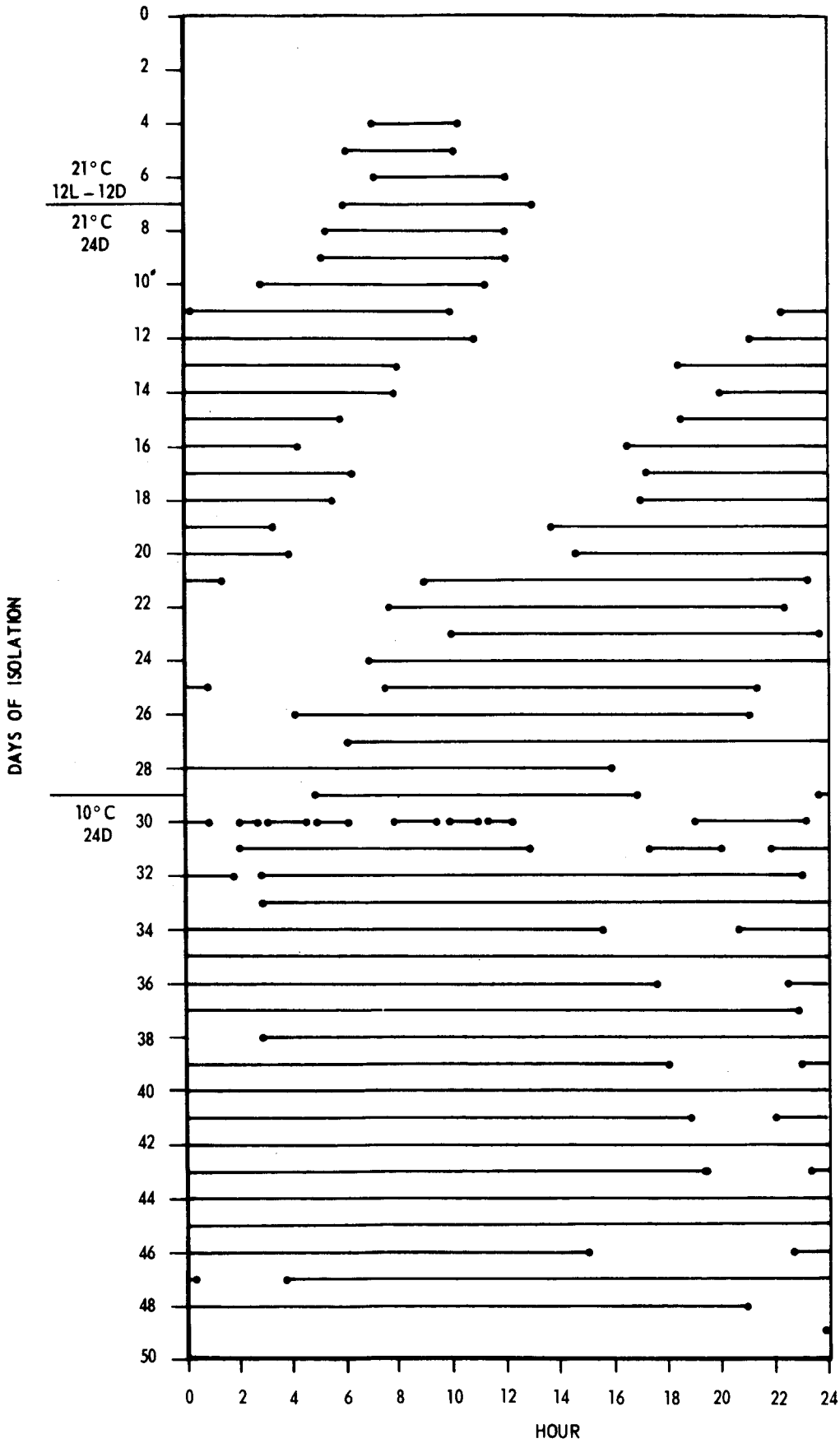
Figure 1 - Torpor periods of Perognathus longimembris maintained with excess food, constant dark, in isolation, during two temperature regimens. The dark bar represents the period of the day in which body temperature was near ambient temperature, and the absence of the bar represents normal body temperature.



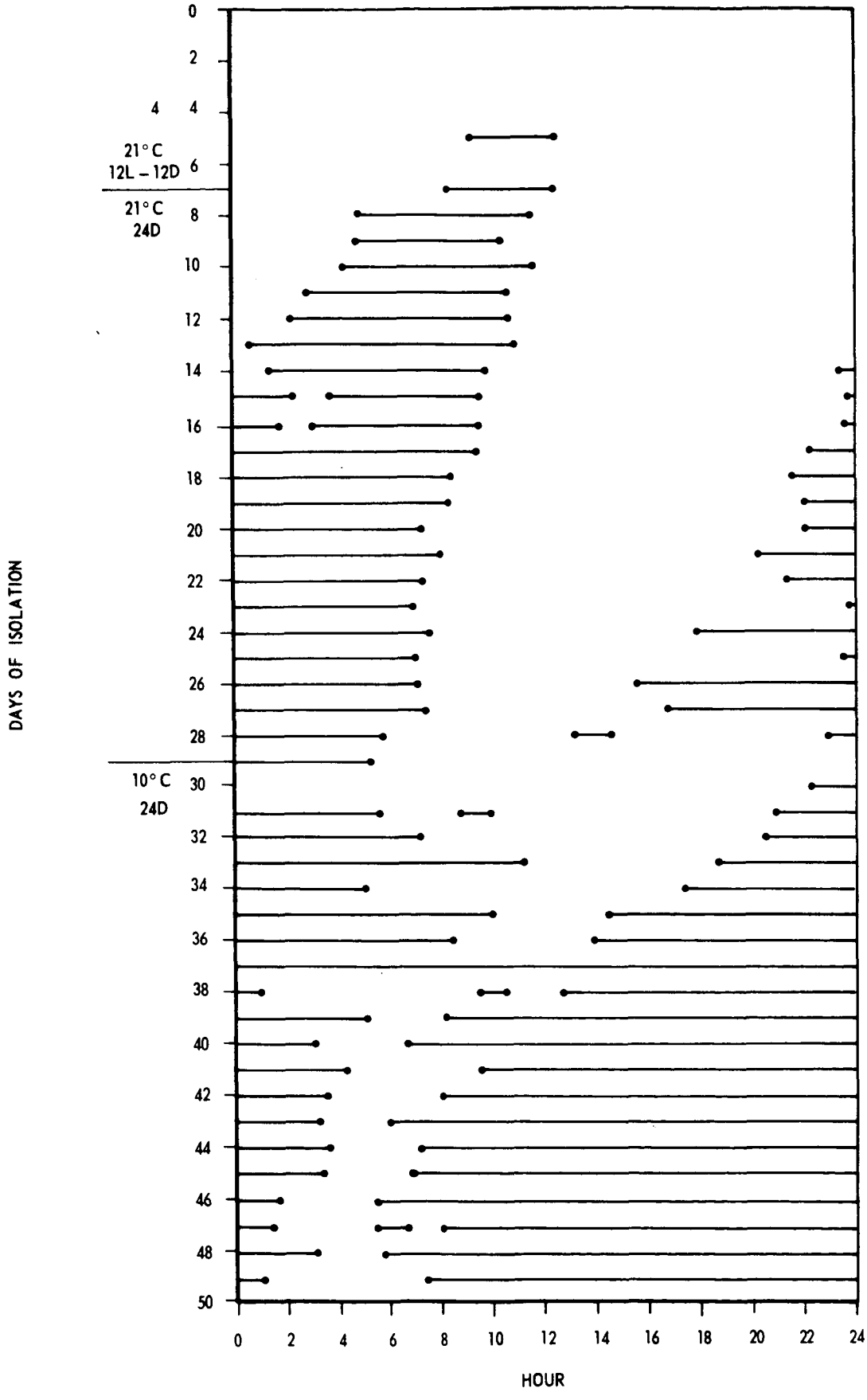
P. longimembris #3 (L-1520 ♀)



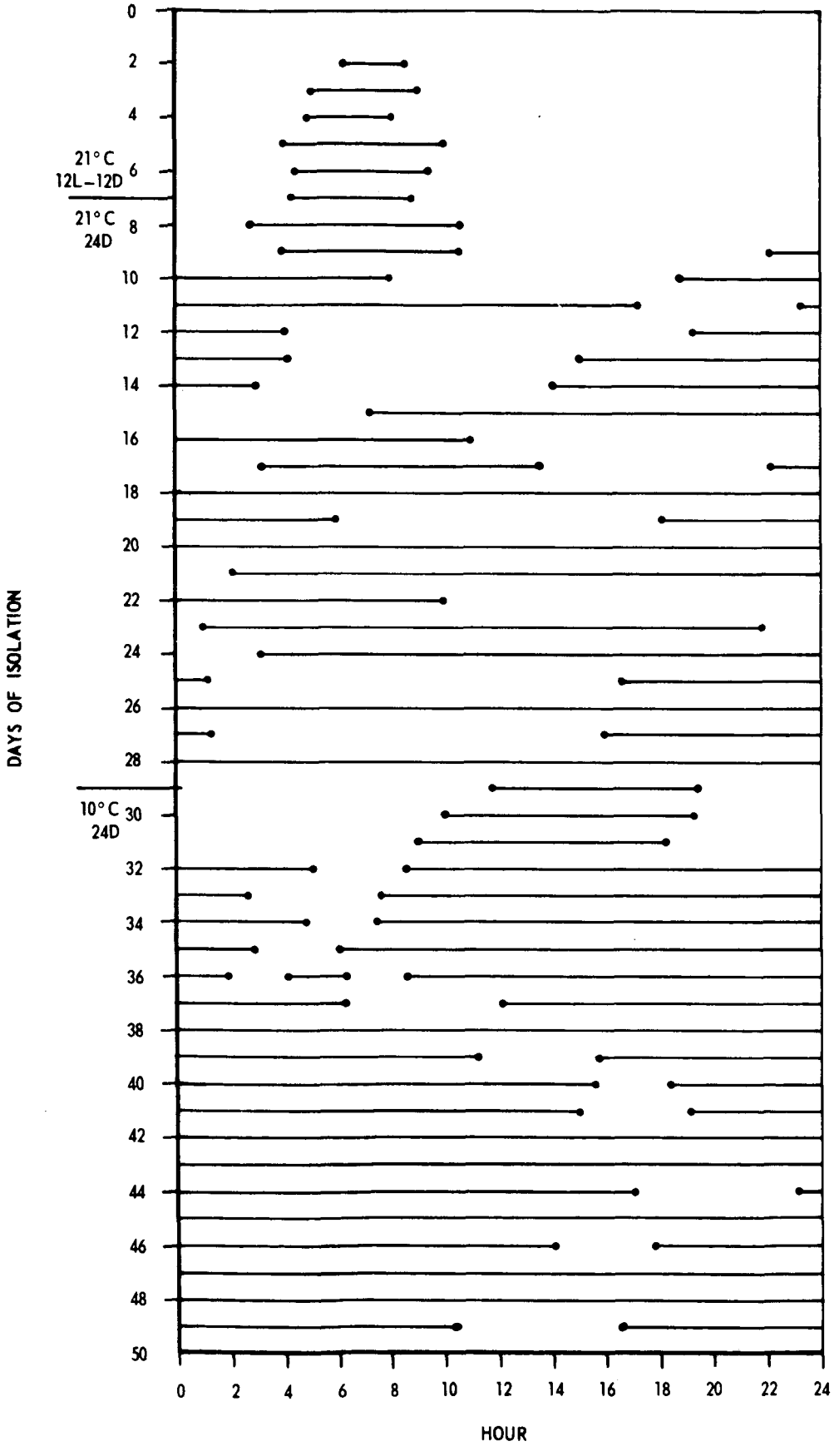
P. longimembris #4 (L-1532 ♂)



P. longimembris #5 (L-1579 ♀)

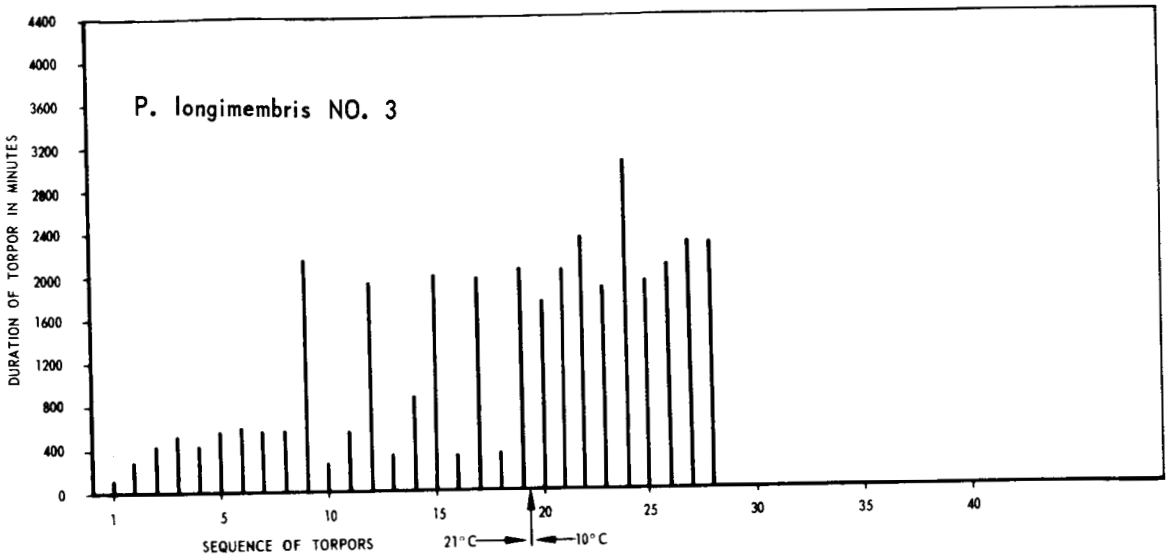


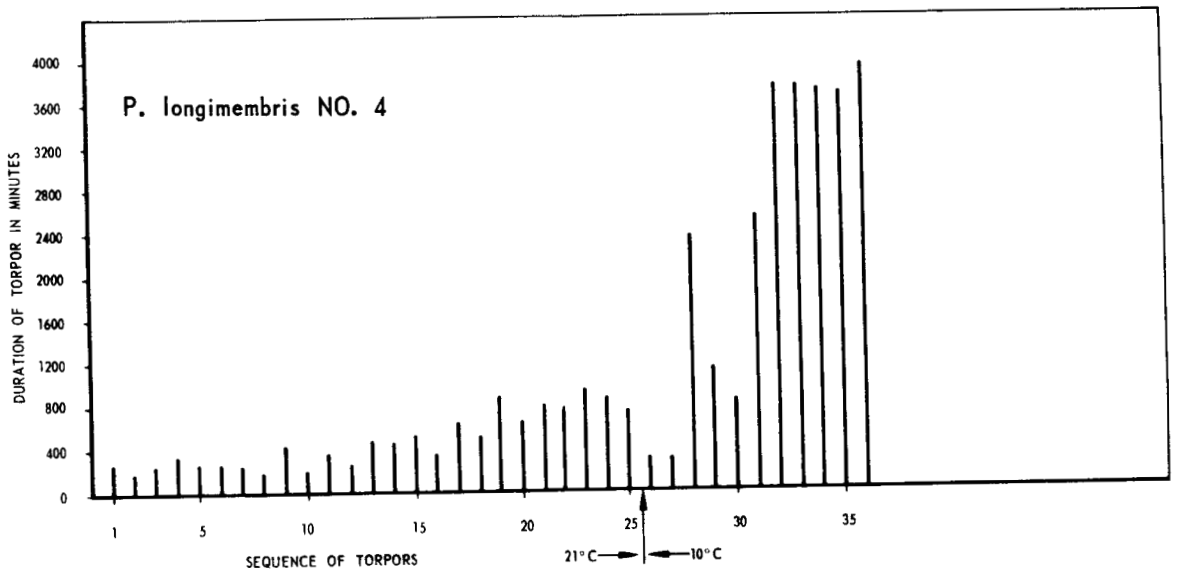
P. longimembris #6 (L-1822 ♀)

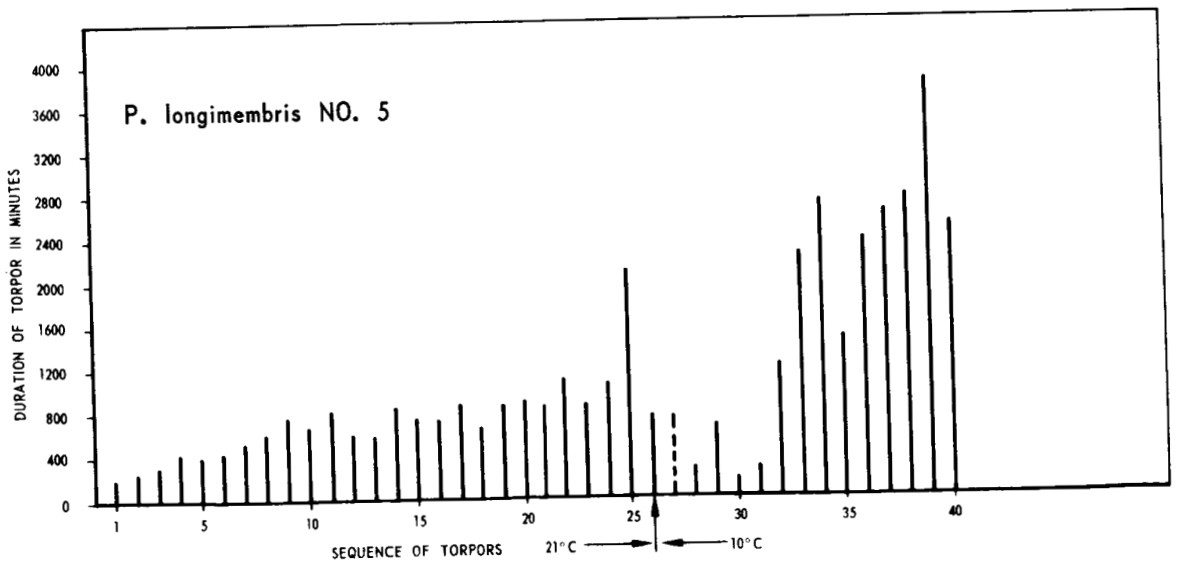


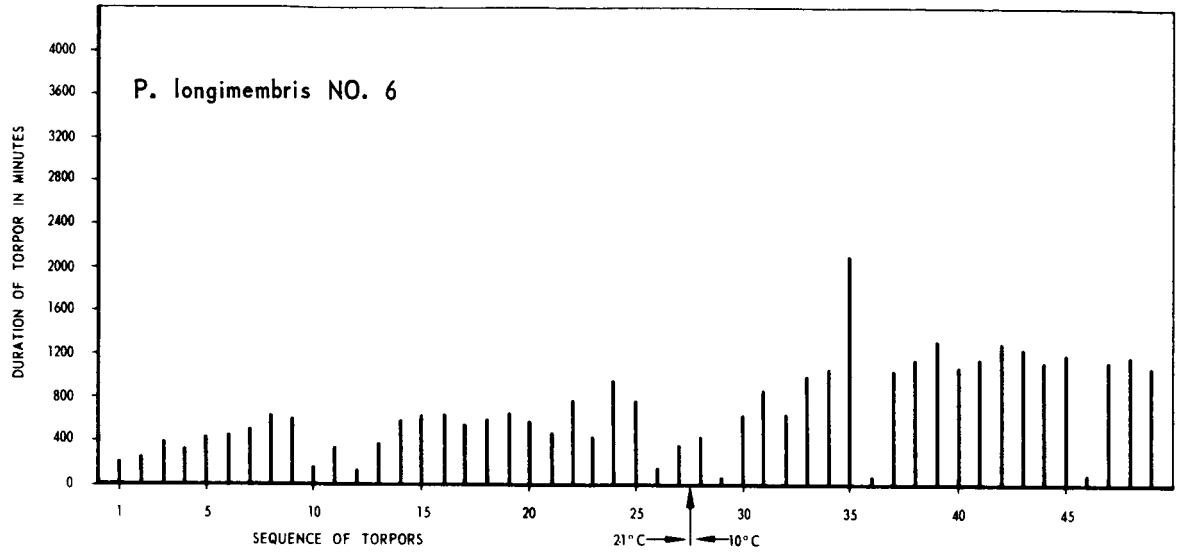
P. longimembris #7 (L-1425 ♀)

Figure 2 - Duration of individual torpor periods of P. longimembris


P. longimembris NO. 3
DURATION OF TORPOR IN MINUTES
4400
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1
5
10
15
20
25
30
35
40
SEQUENCE OF TORPORS
21°C
10°C


P. longimembris NO. 4
DURATION OF TORPOR IN MINUTES
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1
5
10
15
20
25
30
35
SEQUENCE OF TORPORS
21°C
10°C


P. longimembris NO. 5
DURATION OF TORPOR IN MINUTES
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1
5
10
15
20
25
30
35
40
SEQUENCE OF TORPORS
21°C
10°C


P. longimembris NO. 6
DURATION OF TORPOR IN MINUTES
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1
5
10
15
20
25
30
35
40
45
SEQUENCE OF TORPORS
21°C
10°C

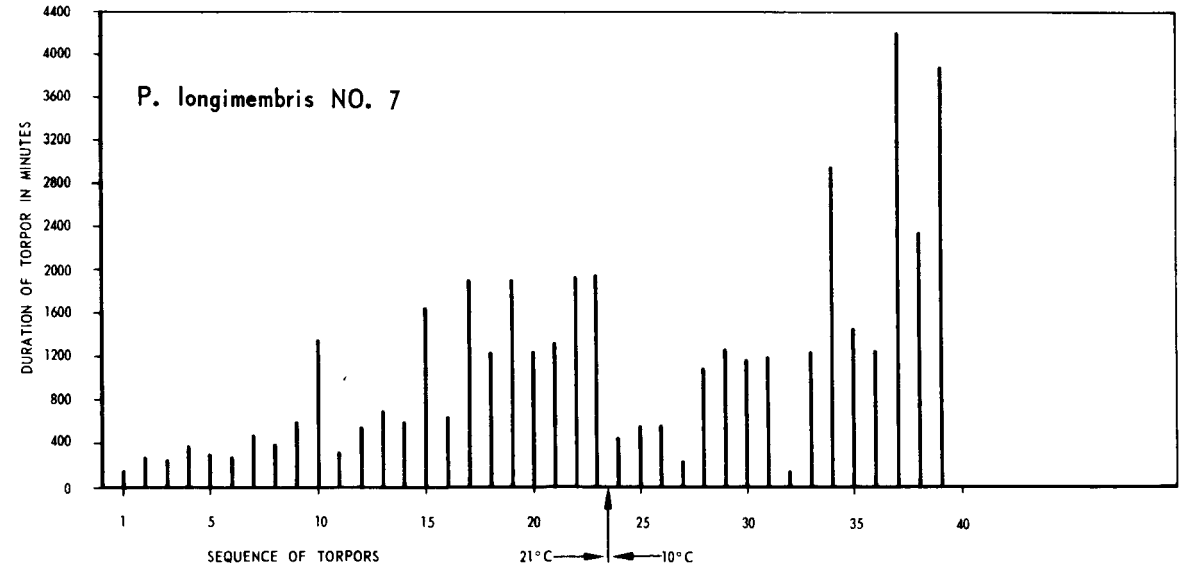

P. longimembris NO. 7
DURATION OF TORPOR IN MINUTES
4400
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1
5
10
15
20
25
30
35
40
SEQUENCE OF TORPORS
21°C
10°C

Figure 3 - The times of entry and arousal from torpor of P. longimembris #4 during two temperature regimens. Note decrease of period length in 10°C ambient and maintenance of accurate rhythm.

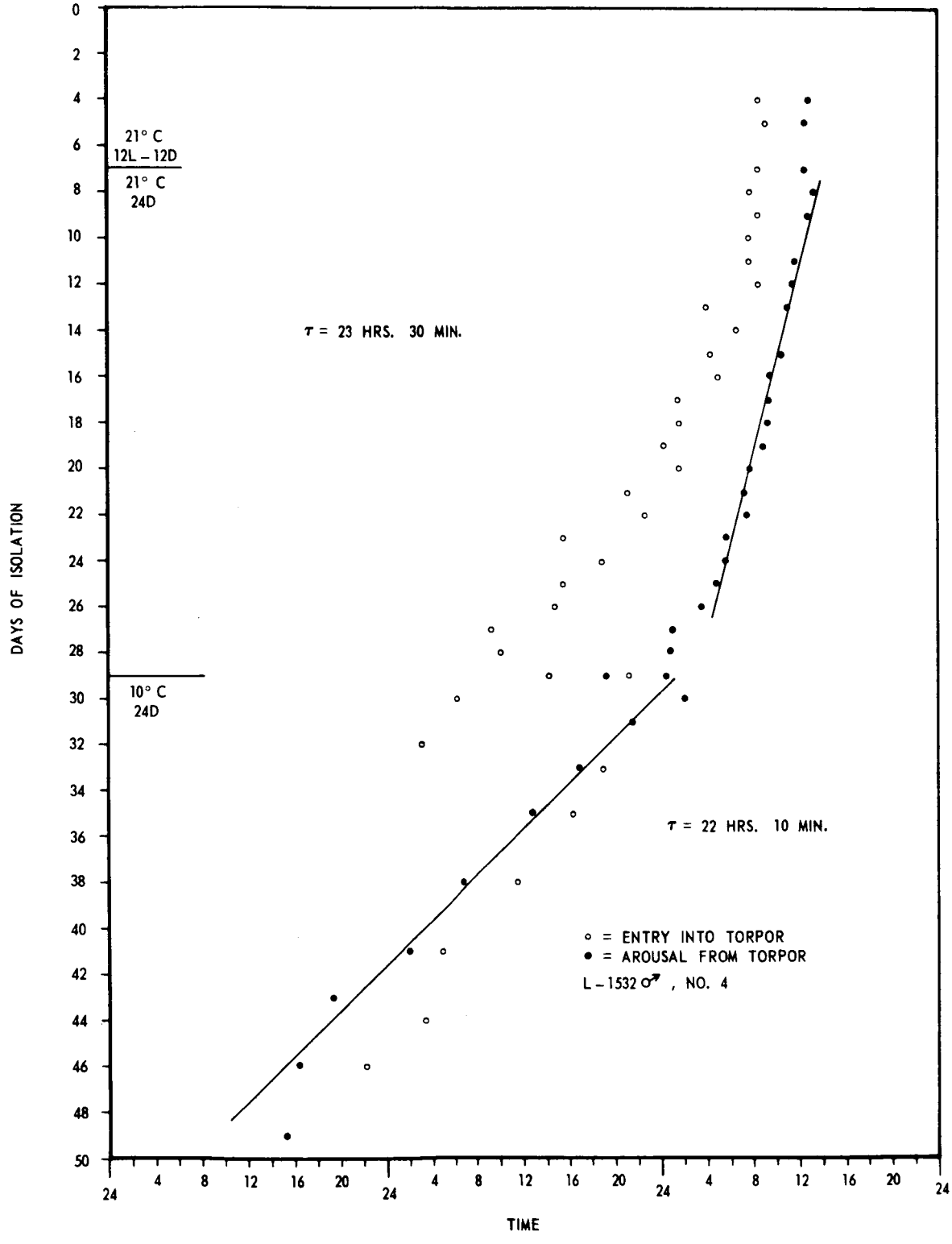
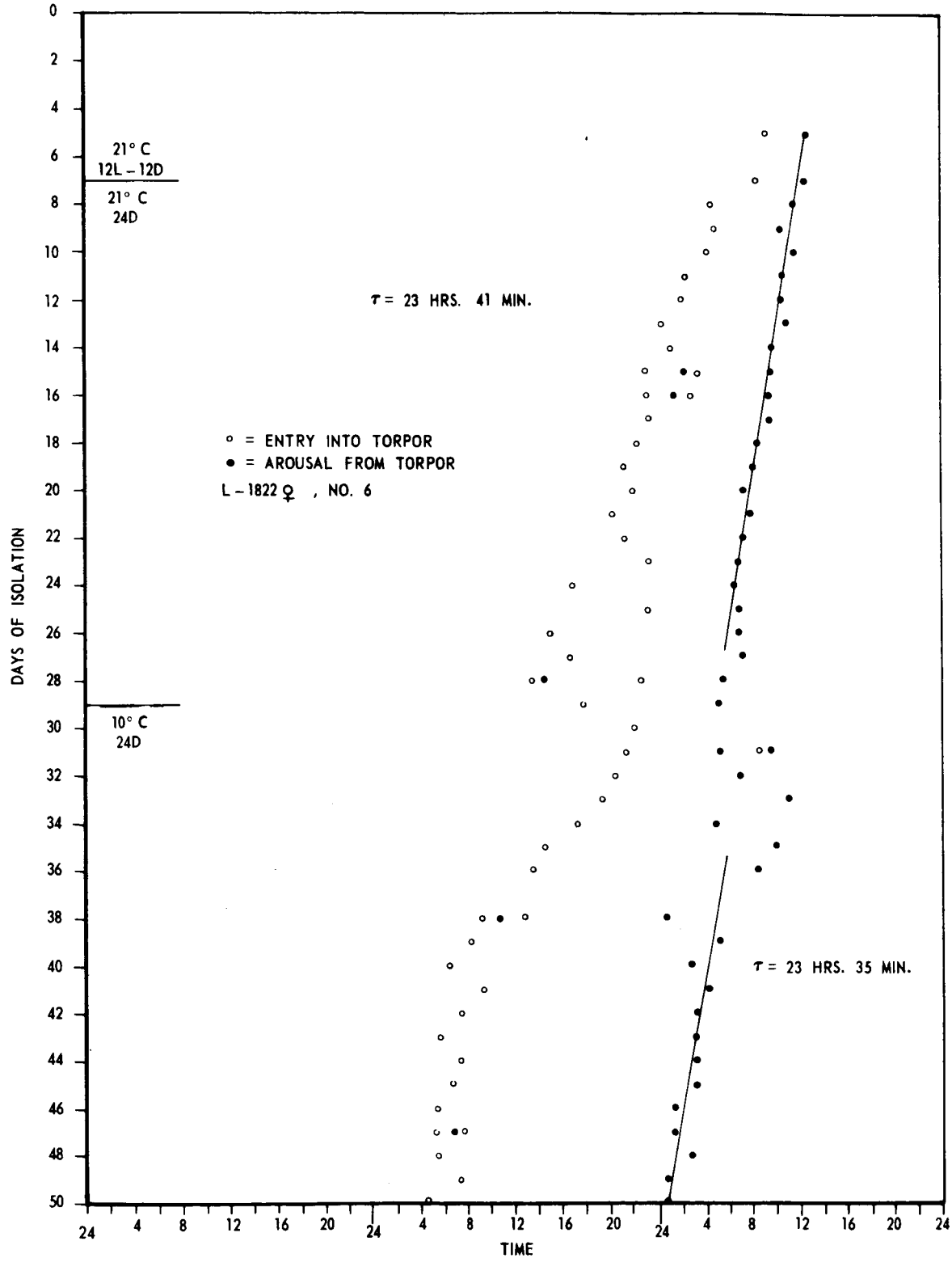

DAYS OF ISOLATION
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
21° C
12L – 12D
21° C
24D
10° C
24D
τ = 23 HRS. 30 MIN.
τ = 22 HRS. 10 MIN.
○ = ENTRY INTO TORPOR
● = AROUSAL FROM TORPOR
L – 1532 ♂ , NO. 4
24
4
8
12
16
20
24
4
8
12
16
20
24
4
8
12
16
20
24
TIME

Figure 4 - The times of entry and arousal from torpor of P. longimembris #6 during two temperature regimens. Note disturbance of rhythm at temperature change and re-establishment after about 10 days.


21° C
12L – 12D
21° C
24D
10° C
24D
τ = 23 HRS. 41 MIN.
τ = 23 HRS. 35 MIN.
° = ENTRY INTO TORPOR
• = AROUSAL FROM TORPOR
L – 1822 ♀ , NO. 6
DAYS OF ISOLATION
TIME
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
24
4
8
12
16
20
24
4
8
12
16
20
24
4
8
12
16
20
24

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